Articles

Bone Marrow Transplantation Beyond Treatment of Aplasia and Neoplasia

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Marrow transplantation has proved to be an important modality in the treatment of aplastic anemia, acute leukemia, and some other malignant diseases of hemopoietic cells. Much less attention has been paid to the role of marrow transplantation in the treatment of stem cell disorders such as sickle cell disease, chronic granulomatous disease, or Gaucher's disease. Allogeneic transplantation is successful in these disorders, but carries a considerable risk to the recipient. Autologous transplantation becomes a reality when efficient gene transfer with sustained expression is developed. As methods are developed to harvest hemopoietic stem cells from the peripheral blood and to amplify these cells without causing them to differentiate, transplantation will become increasingly valuable in the treatment of stem cell disorders.

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The first recorded effort to transplant marrow from one human to another was when Osgood and co-workers in 1939 intravenously infused marrow cells into a patient with aplastic anemia,[1] but it was not until the 1950s that serious attempts were made to implement marrow transplantation as a therapeutic modality. By 1961 bone marrow transplantation had been used in 57 patients to treat acute leukemia.[2] None enjoyed a long remission, and most died within the first month or so of transplantation. Tissue typing had not been developed, and antibiotic and transfusion support was insufficiently advanced to bring many patients through the aplastic phase caused by the preparation for transplantation by total body irradiation.

Successful efforts to transplant marrow had to await understanding of the human leukocyte antigen system and the development of means of supporting patients who had few circulating leukocytes or platelets. In the succeeding decade, however, many advances were made, both in the understanding of transplantation immunology and in the quality of supportive care. By 1974 Thomas and colleagues had transplanted bone marrow to 37 patients with severe aplastic anemia. Although standard therapy offered these patients scarcely any chance of recovery, 17 (nearly 50%) became long-term survivors.[3]

In 1977 Thomas and associates of Seattle, Washington, reported the remarkable response of patients with acute leukemia to allogeneic bone marrow transplantation. Some patients who had far-advanced relapsed acute leukemia survived for long periods of time. The responses were best in those who were in fairly good condition at the time of transplantation. These findings were not generally appreciated at the time, but had a profound effect on my own thinking and professional activities. In the middle 1970s Karl Blume and I established a bone marrow transplantation program at the City of Hope (Duarte, California). We reasoned that if patients with far-advanced leukemia could have a good response to bone marrow transplantation, the response in patients who were in partial or complete remission at the time of transplantation should be even better. The results of marrow transplantation in this clinical setting proved to be extremely gratifying.[5,6] Thomas's group, too, had appreciated the potential of transplantation in better risk patients, and soon they published encouraging results in such patients.[7]

By 1982 several groups began to report encouraging results in the treatment of chronic myelogenous leukemia by transplantation.[8-10] Because of these successes, the vast majority of patients in the 1980s and early 1990s received bone marrow transplants for acute leukemia, chronic granulocytic leukemia, lymphomas, or aplastic anemias (Figure 1).[11] Only a few patients with genetic or metabolic disorders have received transplants, however. I will discuss what I consider to be the potential for helping these patients by bone marrow transplantation. I will also com-

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ABBREVIATIONS USED IN TEXT

G6PD = glucose-6-phosphate dehydrogenase
LTR = long terminal repeat

ment on some recent developments that should improve transplantation in all groups of patients.

Stem Cell Disorders

Although patients with sickle cell disease; thalassemia; hemolytic anemia due to pyruvate kinase, glucose-6-phosphate dehydrogenase (G6PD), or pyrimidine 5′-nucleotidase deficiency; chronic granulomatous disease; hereditary methemoglobinemia; severe combined immunodeficiency due to adenosine deaminase deficiency; and those with Gaucher's disease have different clinical syndromes, they have one thing in common. In all of these patients, their disease is due to a genetically determined protein abnormality in the progeny of hematopoietic stem cells.

Such stem cell disorders can produce symptoms that vary from mild to severe. Glucose-6-phosphate dehydrogenase deficiency and hereditary methemoglobinemia are usually not serious medical problems. On the other hand, there are disorders such as severe combined immunodeficiency and chronic granulomatous disease in which an enzyme defect produces a severe disease and in which the risk of bone marrow transplantation may be clinically justified.

Two approaches to the treatment of hereditary stem cell disorders are allogeneic transplantation and autologous transplantation.

Allogeneic Transplantation for Hereditary Stem Cell Diseases

Among the stem cell defects, the greatest gain in experience in allogeneic transplantation has been gained in thalassemia.[12] A large number of patients, particularly in Italy, have received transplants. The results have been heartening, with the mortality incidental to the procedure about 10%. Of interest, some patients have actually relapsed. We generally regard relapse as a phenomenon associated with leukemia, but in these patients the relapse signifies that the thalassemic marrow re-expanded, crowding out the normal marrow graft.

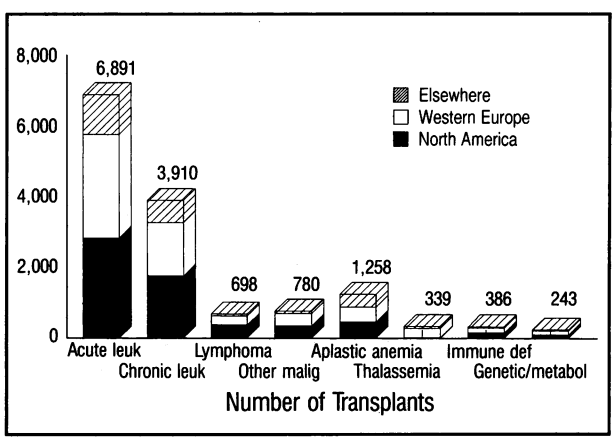

Figure 1.—Indications are shown for allogeneic or syngeneic bone marrow transplantation from a 1988-1990 survey (from Bortin et al[11]; courtesy of D. Van Epps, Baxter Healthcare Corporation). def = deficiency, leuk = leukemia, malig = malignant disease, metabol = metabolic

Transplantation has been implemented only recently in sickle cell disease.[13] Although thalassemia major is almost uniformly fatal when it is not treated, and iron chelation therapy, which may prolong life, is not available everywhere, the course of sickle cell disease is much more difficult to predict and more variable. The first transplantation in a patient with sickle cell disease was carried out in 1984, not to treat sickle cell disease, but rather to treat acute leukemia in a patient who happened also to have sickle cell disease.[14] After transplantation this patient had the sickle cell trait, having sickle and normal hemoglobin levels exactly the same as those found in the donor.

In the past few years many transplantations have been carried out for the treatment of sickle cell disease itself. In Belgium, France, and Italy, 50 patients have received transplants, and an encouraging 41 of them have survived, with a follow-up of between 1 and 75 months.[15-17] In the United States only three patients with sickle cell disease have had transplants. The follow-up is 15 to 18 months, and all three survive. There is a national collaborative protocol for transplantation in children who have suitable risk factors and an HLA-matched donor.*

What is needed in sickle cell disease is a good prognostic marker that would indicate which children should have transplants and which should not. If we knew which children would have a stroke, for example, we would know that they would need transplantation. Marrow transplantation technology has reached the stage where the mortality intrinsic to the procedure is probably less than 10%. As a result, a consensus has developed that, even lacking important prognostic information for each patient, sickle cell disease is a severe enough disorder to justify the risks of allogeneic marrow transplantation in children with a matched family donor.

Autologous Transplantation in the Treatment of Hereditary Stem Cell Diseases

The other type of transplantation, which, of course, avoids graft-versus-host disease, is autologous transplantation—harvesting marrow from a patient and then reinfusing it. Obviously this would not cure genetic diseases if the defect in the reinfused cells were not corrected. As a means to correct the genetic defect, various vectors have been used to insert genes into hematopoietic cells.

The vectors most commonly used are retroviral. Retroviruses are RNA viruses. Their RNA is reverse transcribed into DNA in the infected cell and is inserted in the

*These data were not all published at the time of this presentation, and I am indebted to Keith Sullivan, MD, University of Washington, Seattle, for providing them.

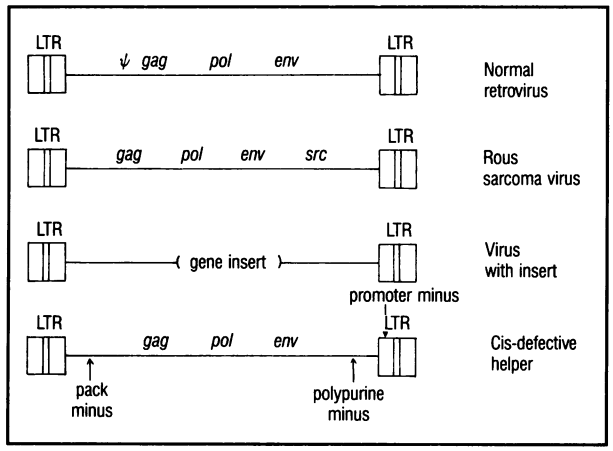

Figure 2.—The diagram shows the anatomy of retroviruses. LTR = long terminal repeat

genome and functions there in a stable manner. The prototype retrovirus is shown at the top of Figure 2. It has a relatively simple structure consisting of two end pieces designated long terminal repeats (LTRs) and three genes—*gag*, a viral glycoprotein, *pol*, the reverse transcriptase or polymerase, and *env* or the envelope gene. Some retroviruses, such as the Rous sarcoma virus, carry oncogenes.

To use retroviruses to transfer genes, the three viral genes are removed from a DNA copy of the gene and are replaced with the gene of interest, sometimes together with a selectable marker like the *neo* gene. Obviously a virus constructed in this manner could not replicate because it possesses none of the genes required for making the envelope, the reverse transcriptase, or the viral proteins. To replicate, such a vector needs a helper virus. The native virus can serve as such a helper. If it were present in the cell, it would produce the gene products that package the RNA of interest into an infectious particle. The problem is that it would also package itself, resulting in continuing infection with a retrovirus. To circumvent this problem, a defective helper, one that has several lesions that prevent it from replicating, is used. For example, a packaging region is required to package the RNA into the virus. If this region is defective in a strand of RNA, it will not be incorporated into a viral particle. The procedure, then, is to transfect a producer line with two viral constructs. One, the helper, has all of the viral genes, making possible the packaging of the gene of interest. The other contains the gene of interest, such as glucocerebrosidase for Gaucher's disease, adenosine deaminase for immunodeficiency, or any of the other genes that one might want to transfer. The virions containing the RNA version of the gene of interest bud from the producer lines and can then be used to infect the target cell. Several years ago Joseph Sorge, MD, in our laboratory used this method to transduce cell cultures from patients with Gaucher's disease. When mutant fibroblasts or lymphoblasts were infected with a retrovirus without the gene of interest, their enzyme activity did not increase. When the cells were infected with a virus that contained glucocerebrosidase complementary DNA, enzyme activity became entirely normal.[18] These cells will grow, replicate, and continue to produce the enzyme.

Lymphoblasts have been transduced fairly successfully in the treatment of children with adenosine deaminase deficiency,[19] but for most stem cell disorders lymphocytes or fibroblasts are not the target cells, but rather hematopoietic stem cells. It is much more difficult to achieve production of an enzyme in a hematopoietic stem cell and its progeny.[18] Marrow can be harvested from a mouse, the cells infected with the retroviral vector, and these cells transplanted into irradiated mice. The mice can then be killed at intervals, and one can then determine whether the gene is present and whether it is active. Serial passage can be done to determine whether a true stem cell has been transduced. Several groups have shown that in mice, the glucocerebrosidase gene does function in their macrophages.[20,21] Kohn and co-workers were able to restore the glucocerebrosidase activity to normal in marrow taken from a patient with Gaucher's disease.[22]

Serious problems attend retroviral transfer, however. First, to be effective in the treatment of most stem cell disorders, the efficiency of transduction must be high. In a disease like Gaucher's disease, thalassemia, or sickle cell disease, it is probably insufficient to correct the defect in a few cells. The defect must be corrected in most or all of the cells. Even if those cells that have been transduced are selected, it is not safe to select just 10% or 20% of the cells and administer those; a large number of stem cells is required for successful transplantation. Until recently most vectors did not provide highly efficient transduction. Second, the LTR, the promoter of retroviruses, does not provide tissue-specific regulation, which is required for some genes. For example, if hemoglobin genes are transferred into hematopoietic stem cells, they should be expressed only in erythrocytes, not in granulocytes or lymphocytes. This problem can be circumvented. In the case of hemoglobin, the locus control region seems to provide specificity for the hemoglobin gene.[23] This problem needs to be addressed in most cases of gene transfer.

The continued expression of transduced genes is a major problem when retroviruses serve as vectors. This difficulty seems to have been overcome in mice, but in primates it is more of an obstacle. Primitive cells seem to have evolved a mechanism that defends them against retroviruses by inactivating them.[24] If a viral gene does not continue to be expressed for many months and years, then the long-term aims of the procedure have not been fulfilled.

Finally, there is the lingering risk of malignant transformation. This risk is the consequence of the fact that a retrovirus is inserted into a genome in random locations. If insertion happens to be next to a regulatory gene, it may result in uninhibited proliferation of that cell. When monkeys were infected with a virus preparation that was not helper-free, lymphomas developed.[25] The risk of such transformation is minimized by using vectors that are helper-free—that is, vectors that cannot themselves proliferate. With the use of such vectors, tumors have not been observed.

New Technologies

In transplanting marrow cells, it is our aim to transfer into a patient a self-renewing cell that is capable of differentiating into erythrocytes, leukocytes, granulocytes, monocytes, and platelets. The transplantation of a cell that has already started along this pathway will result in the eventual differentiation of the progeny of the transplanted cell into a cell without renewal potential, and the graft will ultimately fail.

In the past few years we have been aware that among the bone marrow nucleated cells, a small subset of cells express the CD34 antigen on their surface. Members of this subset apparently have many of the properties of stem cells in that they are able to differentiate into all of the different lineages. Technology has now been developed that makes it possible to select these cells on a sufficient scale to transplant them. One such method uses a magnetic bead coated with an antibody against CD34. Beads are mixed with the blood or marrow, and the CD34 cells adherent to the beads are separated magnetically and are then freed from the beads by cleaving the CD34 with chymopapain. This purifies CD34 progenitor cells and, it is hoped, stem cells. An important next step not yet fully worked out is to stimulate this cell to replicate so that one cell can be expanded to 1×10^3 or 1×10^6. It is important that the amplification process does not cause differentiation. As we better understand the complex network of cytokines that regulate both the proliferation and differentiation of hematopoietic cells, such expansion without differentiation may become possible.

Another new technology consists of the transplantation of adult marrow cells into a fetus with a hereditary disorder. This approach may be successful because the fetus is immunologically naive, and therefore graft rejection is less likely. The results of trials in sheep have been encouraging,[26] and a trial in patients with chronic granulomatous disease is being planned but has not yet been implemented.

Marrow Transplantation in the 21st Century

I envision bone marrow transplantation in the 21st century as a procedure in which we will no longer actually transplant marrow. Rather, we will isolate stem cells from the peripheral blood and amplify them. In the 21st century, we will likely no longer speak of marrow transplantation but of stem cell transplantation. In the next decade, we should see more genetic diseases that are expressed in the progenitors of hematopoietic stem cells corrected by gene transfer followed by stem cell transplantation.

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